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Glatiramer acetate blocks the activation of THP-1 cells by interferon-γ

Qingdi Li [a,b,c,d,2], Ron Milo [a,d,3], Hillel Panitch [a,d], Peggy Swoveland [a,d], Christopher T. Bever Jr. [a,b,d,*]

[a] Department of Neurology, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[b] Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[c] Department of Microbiology, University of Maryland School of Dentistry, Baltimore, MD 21201, USA
[d] Medical Research Service, Baltimore VA Medical Center, Baltimore, MD 21201, USA

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Abstract

Glatiramer acetate (previously known as copolymer 1) is a synthetic copolymer of four amino acids that has been approved for use in the treatment of multiple sclerosis. It has been shown to suppress myelin antigen specific T cell activation by competing with these antigens at the major histocompatibility complex class II binding site and by inducing antigen specific suppressor T cells. In this study we investigated the effects of glatiramer acetate on the human monocytic cell line, THP-1, activated by lipopolysaccharide and interferon-γ as a model for macrophages. At non-toxic concentrations of glatiramer acetate there were dose dependent reductions in the percentage of cells expressing human leukocyte DR and DQ antigen as well as in mean fluorescence intensity by flow cytometry. Production of tumor necrosis factor-α and the lysosomal cysteine proteinase cathepsin B were markedly inhibited, but production of interleukin-1 increased. These results suggest that glatiramer acetate might alter macrophage effector function and suggest that further studies in human monocytes and macrophages are warranted. Published by Elsevier Science B.V.

Keywords: Glatiramer acetate; HLA Class II; Cytokine; Cathepsin B; THP-1 cells; Multiple sclerosis

1. Introduction

Glatiramer acetate (previously called copolymer 1) is a synthetic basic copolymer of four amino acids (alanine, lysine, glutamic acid, and tyrosine) (Teitelbaum et al., 1971) that cross-reacts with myelin basic protein. It has been shown that glatiramer acetate can suppress clinical and pathological signs of experimental autoimmune encephalomyelitis induced by myelin basic protein (Arnon and Teitelbaum, 1988), proteolipid protein (Teitelbaum et al., 1996) or myelin oligodendrocyte glycoprotein (Rosbo et al., 1996) and reduce the exacerbation rate in relapsing–remitting multiple sclerosis in double-blind, placebo-controlled clinical trials (Johnson et al., 1995). The mechanism of activity, however, is not fully understood. Previous studies indicated that glatiramer acetate specifically inhibits the T cell response to myelin basic protein in vitro by competing with it at the major histocompatibility complex class II binding site (Teitelbaum et al., 1992). It can also induce antigen-specific suppressor T cells in vivo that prevent development of experimental autoimmune encephalomyelitis and suppress myelin basic protein specific T cell activation (Aharoni et al., 1993). Human peripheral blood mononuclear cells often exhibit blastogenic responses to glatiramer acetate suggesting T cell recognition (Brosnan et al., 1985). However, little data is available on the effects of glatiramer acetate on antigen presentation by monocytes and macrophages, cells which are important in the pathogenesis of autoimmune diseases including multiple sclerosis (Prineas and Graham, 1981). Therefore, in this study we investigated the effects of glatiramer acetate on major histocompatibility complex class II molecule expression, synthesis of a lysosomal

* Corresponding author. Tel.: +1-410-6057061; fax: +1-410-6057937.
[1] These authors contributed equally to this work.
[2] Present address: Clinical Pharmacology Branch, Medical Ovarian Cancer Section, Developmental Therapeutics Department, Division of Clinical Sciences, National Cancer Institute, National Institutes of Health, Bldg. 10, Rm. 13N248, Bethesda, MD 20892, USA.
[3] Present address: Department of Neurology, Assaf Harofeh Medical Center, Zerifin 70300, Israel.

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cysteine proteinase cathepsin B which is implicated in demyelination (Yanagisawa et al., 1984) and production of the monocytic proinflammatory cytokines, tumor necrosis factor-α and interleukin-1β, using the THP-1 cell line. This line is a human monocytic leukemia cell line with characteristics of macrophages including immunological functions, which serves as a useful model for studying the role of monocyte-macrophages in the human immune response (Auwerx, 1991). THP-1 cells can be induced to differentiate into macrophage-like cells by phorbol esters (Li and Bever, 1996) or by bacterial lipopolysaccharide (Li et al., 1997), and can be activated by interferon-γ (Li et al., 1994, 1997; Li and Bever, 1996). These macrophage-like cells express large amounts of major histocompatibility complex molecules (Li et al., 1994), produce large quantities of lysosomal proteinases (Li and Bever, 1996; Li et al., 1997) and secrete typical macrophage-associated cytokines including interleukin-1β and tumor necrosis factor-α (Li et al., 1994), which may play important roles in the pathogenesis of inflammation and demyelination in multiple sclerosis (Selmaj and Raine, 1988; Durum and Oppenheim, 1989). In this report, we present results showing that glatiramer acetate could regulate these functions in our system in vitro.

2. Materials and methods

2.1. Drugs and reagents

Recombinant human interferon-γ was purchased from Genzyme (Cambridge, MA). Lipopolysaccharide (*E. coli*, 0111: B4), *N*-carbobenzoxy-(DL)-L-alanyl-L-arginyl-L-arginine-4-methoxy-β-naphthylamide, α-naphthylamide, brij 35, fast garnet, and trypan blue solution (0.4%) were purchased from Sigma Chemical (St. Louis, MO). Glatiramer acetate, composed of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a residual molar ratio of 4.2:1.4:3.4:1.0 (Batch #29035) was a gift from Teva Pharmaceutical (Petah-Tiqva). The THP-1 cell line was obtained from the American Type Culture Collection (Rockville, MD). RPMI 1640 medium, phosphate buffered saline (PBS), penicillin-G, streptomycin, L-glutamine, sodium pyruvate were purchased from Life Technologies (Gibco, Grand Island, NY). Fetal bovine serum was obtained from HyClone (Logan, UT).

2.2. Cells and cell culture

THP-1 cells were derived from a 1-year-old individual with acute monocytic leukemia and have monocyte-macrophage characteristics (Tsuchiya et al., 1980). These c lls were grown in 75 cm^2 plastic tissue culture flasks (Corning, Corning, NY) in 20 ml of RPMI 1640 medium supplemented with the antibiotics penicillin-G (50 U/ml) and streptomycin (50 μg/ml), 10% (v/v) heat-inactivated fetal bovine serum, L-glutamine (2 mM), *N*-2-hydroxyet ylpiperazine-*N*'-2-ethanesulfonic acid buffer (HEPES) (mM) (GIBCO BRL, Gaithersberg, MD), pH 7.3 (cRPM sodium pyruvate (1 mM), in logarithmic growth at 37°C a humidified atmosphere consisting of 5% CO_2: 95% a Cells were routinely tested for mycoplasmal infection u ing a commercial assay system (MycoTect; GIBCO BRI and new cultures were established monthly from froz stocks. All media and reagents contained < 0.1 ng/ endotoxin as determined by *Limulus polyphemus* amet cyte lysate assay (Whittaker Bioproducts, Walkersvil MD). Cell viability was determined by trypan blue d exclusion. Before starting the experiments, the cells we grown for 1 day after subculturing. Subculturing was do by harvesting the cells, resuspending in cell culture medi and culturing them at 5×10^5 cells/ml. Differentiation THP-1 cells was assessed by observing adh rence and c morphology. Cells were incubated with lipopolysacchari and where indicated, glatiramer acetate for 24 h, then t medium was removed and replaced with fresh medi containing interferon-γ. The cells were harvested for u in the cathepsin B assay 48 h later. Interferon-γ was us at a saturating dose of 500 U/ml in these experiments a lipopolysaccharide was used at a dose of 25 μg/ml unl otherwise indicated.

2.3. Flow cytometric analysis

THP-1 cells were pretreated with various concentratio of lipopolysaccharide, interferon-γ, or glatiramer acet as described. After an incubation period, cells were h vested, washed twice with phosphate buffered saline (PB counted, and resuspended at 5×10^5 viable cells per 50 of PBS supplemented with 2% fetal bovin serum a 0.1% sodium azide in 5 ml polypropylene tubes. Sampl were then stained with 5 μl of phycoerythrin-conjugat mouse anti-human leukocyte antigen-DR or fluoresce isothiocyanate (FITC)-conjugated mouse anti-hum leukocyte antigen-DQ or the appropriate isotype contr (Olympus, Lake Success, NY). After incubation on ice f 45 min, cells were washed twice, fixed with 0.5 ml 0.5% paraformaldehyde, and analyzed for percentage human leukocyte antigen-DR or human leukocyte antige DQ positive cells and mean fluorescence intensity in Epics Elite flow cytometer (Coulter, Hialeah, FL). Ga were adjusted to include 100% of the viable cells, a 5000 cells per sample were counted.

2.4. Cytokine assays

The culture supernatants w re collected after 48 h incubation under the conditions described. The quantiti of these cytokines were measured using human tum necrosis factor-α or interleukin-1β enzyme-linked i munosorbent assay (ELISA) kits from R + D Systen (Minneapolis, MN); the detection limits for these kits we

15 pg/ml for tumor necrosis factor-α and 10 pg/ml for interleukin-1β, respectively. The antibodies in these kits did not have any detectable cross-reactivity with other antigens. In accordance with the manufacturer's instructions the samples were incubated in duplicate wells and read on an automated ELISA reader (Anthos-Labtec Instruments, Salzburg).

2.5. Cathepsin B assay

A standard assay based on the release of naphthalamine from an artificial substrate was used (Barrett and Kirschke, 1981). Stimulated THP-1 cells were harvested by gentle scraping with a disposable rubber cell scraper (Costar, Cambridge, MA). Cells were washed 3 times with PBS (pH 7.0), counted, and 5×10^5 cells were aliquoted for assay. Cells were resuspended in PBS (pH 6.0) and lysed by freeze-thawing and sonication 3 times. The resulting lysate was then assayed in triplicate by a previously described assay based on the release of naphthylamine from an artificial substrate (Bever et al., 1989). In brief, aliquots of supernatants prepared by centrifugation were added to activation buffer to give 250 μl containing 100 mM phosphate and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 6.0. After 5 min, *N*-carbobenzoxy-(DL)-L-alanyl-L-arginyl-L-arginine-4-methoxy-β-naphthylamide (40 mg/ml), a substrate not degraded by cathepsin H and L (Barrett and Kirschke, 1981) in dimethyl sulfoxide (DMSO) was added. The mixture was incubated at 37°C for 50 min, then the reaction was stopped with the addition of the color reagent, mersalyl-EDTA in Brij 35 with Fast Garnet. After mixing well, the mixture was left at room temperature for approximately 10 min for color development. The samples were read on a spectrophotometer (Stasar II; Gilford, Oberlin, OH) at 520 nm. The assays were standardized with α-naphthylamide and results expressed as nanomoles of naphthalamine released per million cells or mg protein in the lysate.

3. Results

3.1. The effect of glatiramer acetate on human leukocyte antigen-DR and -DQ expression on THP-1 cells

As shown by flow cytometric analysis, 66% of unstimulated THP-1 cells were positive for human leukocyte antigen-DR, and 10% for human leukocyte antigen-DQ (Fig. 1A and B; Table 1). Stimulation of the cells with 25 μg/ml of lipopolysaccharide for 24 h follow d by 500

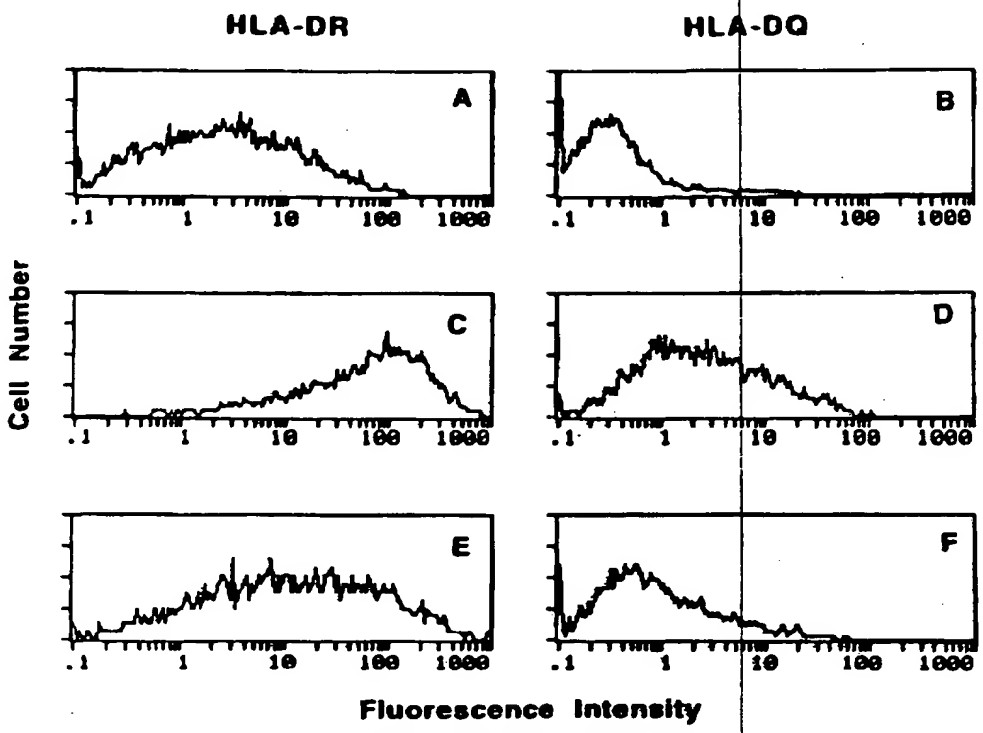

Fig. 1. The effect of glatiramer acetate on cell surface human leukocyte antigen-DR and human leukocyte antigen-DQ expression on THP-1 cells. THP-1 cells cultured for 72 h in the absence of lipopolysaccharide, interferon-γ and glatiramer acetate (A and B). THP-1 cells cultured for 24 h in the presence of 25 μg/ml lipopolysaccharide followed by 48 h with 500 U/ml interferon-γ (C and D). THP-1 cells cultured with 25 μg/ml lipopolysaccharide and 20 μg/ml glatiramer acetate for 24 h followed by 500 U/ml interferon-γ for 48 h (E and F). The cells were then analyzed by flow cytometry for the molecules indicated, as described in Section 2.

Table 1
The effect of glatiramer acetate on human leukocyte antigen-DR and human leukocyte antigen-DQ expression on THP-1 cells[a]

Treatment[b]	HLA-DR		HLA-DQ	
	positive[c] (%)	fluorescence[d] (mean)	positive[c] (%)	fluorescence[d] (mean)
None	66	2.5	10	0.37
LPS + IFN-γ	96	53.0	44	2.46
(LPS + 5 μg/ml Cop 1) + IFN-γ	89	40.4	30	2.31
(LPS + 10 μg/ml Cop 1) + IFN-γ	93	38.8	39	2.23
(LPS + 20 μg/ml Cop 1) + IFN-γ	76	11.6	19	0.97

[a]Representative fluorescence profiles are given in Fig. 1.
[b]Cells incubated for 24 h in media containing 25 μg/ml of lipopolysaccharide (LPS). Medium was then removed and replaced with fresh mediu containing 500 U/ml of interferon-γ (IFN-γ). Cells were harvested after 48 h of incubation. Glatiramer acetate (Cop 1) was added to the first incubati at the concentrations given.
[c]Percent positive cells relative to the irrelevant antibody control.
[d]Mean fluorescence intensity of the positive cells.

U/ml of interferon-γ for 48 h upregulated the expression of human leukocyte antigen-DR and -DQ (Fig. 1C and D). As shown in Table 1, the percentage of positive cells increased to 96.4% and 44%, respectively, after treatment with lipopolysaccharide and interferon-γ. Incubation of the cells with 20 μg/ml glatiramer acetate for 24 h decreased the number of stained cells to 76% and 19%, respectively (Fig. 1E and F; Table 1). Untreated cells showed low mean fluorescence intensity values for human leukocyte antigen-DR and -DQ (Table 1). Lipopolysaccharide and interferon-γ additively increased the density of these cell surface molecules (data not shown). Treatment of the THP-1 human monocytes in culture with lipopolysaccharide and interferon-γ caused striking increases in both human leukocyte antigen-DR and human leukocyte antigen-DQ mean fluorescence intensity (Table 1). Addition of glatiramer acetate to the stimulated cells inhibited class human leukocyte antigen expressions by up to 80% in concentration-dependent manner (Fig. 1E and F; Table 1

3.2. The effect of glatiramer acetate on cytokine production and cathepsin B activity in THP-1 cells

Production of two major macrophage cytokines, interleukin-1β and tumor necrosis factor-α, was measure Unstimulated cells produced nearly undetectable levels both substances, while large amounts were induced b stimulation with lipopolysaccharide and interferon-γ (Fi 2). Glatiramer acetate at a non-toxic concentration of 2 μg/ml inhibited tumor necrosis factor-α production b 80% (Fig. 2), but stimulated the production of interleuki 1β by approximately 50% (Fig. 2). The inhibition tumor necrosis factor-α production by up to 60% w confirmed also in a bioassay using L929 cells (data n shown). The effect of interferon-γ on the intracellul

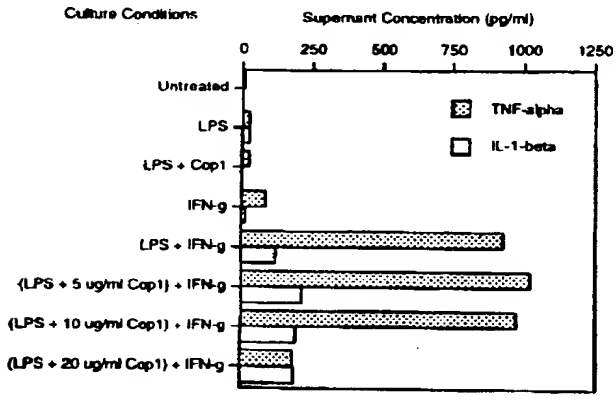

Fig. 2. The effect of glatiramer acetate (cop 1) on the production of tumor necrosis factor-α and interleukin-1β in lipopolysaccharide-primed THP-1 cell cultures by interferon-γ. Cells were incubated with lipopolysaccharide (25 μg/ml) in the presence or absence of glatiramer acetate (cop 1) for 24 h, the medium was changed to fresh medium containing interferon-γ (500 U/ml) for an additional 24 h and supernatants were then collected and assayed for tumor necrosis factor-α and interleukin-1β as described in Section 2. IL-1-beta, interleukin-1β; Cop 1, glatiramer acetate; LPS, lipopolysaccharide; TNF-alpha, tumor necrosis factor-α; IFN-g, interferon-γ.

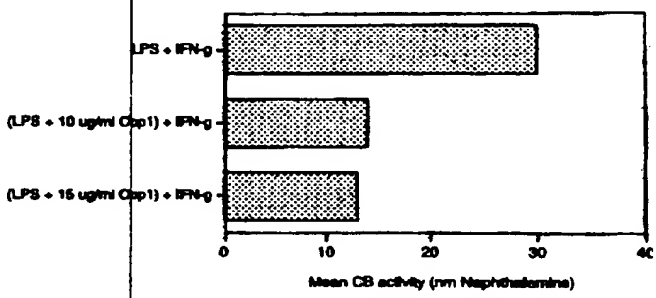

Fig. 3. The effect of glatiramer acetate (cop 1) on cathepsin B activity THP-1 cells. Cells were seeded at a density of 5×10^5 cells/ml in 75 cm flasks and cultured for 24 h in the presence of 25 μg/ml of lipopol saccharide. The medium was then changed to fresh medium containir 500 U/ml of interferon-γ and cells were incubated for an additional 4 h. Cells were then harvested and assayed for cathepsin B activity described in Section 2. Glatiramer acetate (cop 1) at the indicate concentrations was added during the initial incubation time. The resul are the mean of triplicate determinations. IFN-g, interferon-γ; CI cathepsin B; LPS, lipopolysaccharide; Cop 1, glatiramer acetate.

activity of an inducible lysosomal cysteine proteinase, cathepsin B, was also studied using the human monocytic cell line, THP-1. We have shown previously that there were basal low levels of cathepsin B in unstimulated cells. Stimulation of lipopolysaccharide-primed THP-1 cells with interferon-γ increased cathepsin B activity. The effect of interferon-γ was dose- and time-dependent (Li et al., 1997). Incubation with glatiramer acetate for 24 h effectively blocked the interferon-γ-induced increase in monocyte cathepsin B activity (Fig. 3). The toxicity of glatiramer acetate concentrations from 2.5 to 150 μg/ml was examined by cell recovery, trypan blue dye exclusion, and ^{51}Cr release cytotoxicity assay (data not shown) and concentrations of 50 μg/ml and above were found to be toxic. The concentrations of glatiramer acetate used in these experiments were not toxic.

4. Discussion

In this report, we used THP-1 cells as a model of human monocytes and macrophages and studied the effect of glatiramer acetate on the modulation of expression of major histocompatibility complex class II molecules and synthesis of cytokines and cathepsin B. We demonstrated here for the first time that glatiramer acetate downregulates lipopolysaccharide and interferon-γ-induced expression of human leukocyte antigen-DR and -DQ in THP-1 human monocytic/macrophage-like cells. Glatiramer acetate also effectively blocked the interferon-γ-induced increase in tumor necrosis factor-α production and cathepsin B activity, but upregulated the production of interleukin-1β by approximately 50% in lipopolysaccharide-primed THP-1 cells. These results differ from those in a previously published study in which we found no effect (Milo and Panitch, 1995), but those studies differed significantly from the present studies in that the cell line was a B lymphocyte transformed by Epstein-Barr virus infection and only the effect of glatiramer acetate on unstimulated class II antigen expression was examined. These differences suggest that future studies should examine human monocytes and macrophages.

Activated monocytes and macrophages are important in the pathogenesis of multiple sclerosis. Macrophages are found in lesions, actively phagocytizing myelin lamella and ingesting immunoglobulins bound to myelin (Prineas and Graham, 1981). Peripheral blood monocytes are activated in multiple sclerosis and exhibit augmented release of arachidonic acid metabolites (Merrill et al., 1989), toxic oxygen intermediates (Fisher et al., 1988), hydrolytic enzymes (Cieri and Bever, 1990; Bever et al., 1994), and cytokines (Merrill et al., 1989) compared to cells of normal individuals. Monocytes within multiple sclerosis plaques in the brain are also activated and express major histocompatibility complex class II molecules (Traugott et al., 1983). These studies together suggest that activated macrophages are important in multiple sclerosis pathogenesis.

The mechanism of action of glatiramer acetate, which was developed to prevent the development of xperimental allergic encephalomyelitis, is not known. Glatiram r acetate suppresses acute experimental autoimmune encephalomyelitis in various animal species (Teitelbaum et al., 1971; Sela et al., 1990) and chronic-relapsing experimental autoimmune encephalomyelitis in guinea pigs (Keith et al., 1979), and reduced relapse rate and improved disability in relapsing-remitting multiple sclerosis on a phase III multicenter, double-blind, placebo-controlled clinical trial (Johnson et al., 1995). Two possible mechanisms were proposed for the activity of glatiramer acetate in experimental autoimmune encephalomyelitis and multiple sclerosis: (i) induction of antigen-specific suppressor cells (Aharoni et al., 1993); and (ii) competition with myelin antigens for activation of antigen specific T cell lines (Teitelbaum et al., 1992, 1996; Milo and Panitch, 1995; Rosbo et al., 1996). Evidence showed that glatiramer acetate can inhibit the response to myelin basic protein of various myelin basic protein-specific T cell lines only in the presence of antigen-presenting cells, indicating that the site of action of glatiramer acetate is probably on the maj r histocompatibility complex class II molecules (Teitelbaum et al., 1992). Racke et al. (1992) have reported that glatiramer acetate can suppress responses of murine T cells to antigens other than myelin basic protein and postulated that blockade of antigen presentation was related to the restriction element involved rather than to the specific antigen. Together, these studies suggest that one of the possible mechanisms of inhibition of glatiramer acetate is to interfere with antigen presentation which in turn interferes with T cell activation.

It is generally accepted that processing of a protein antigen by an antigen-presenting cell and its presentation as a complex with self-major histocompatibility compl x molecules are prerequisites for antigen recognition by T lymphocytes via their T cell receptors, leading to T cell activation. CD4$^+$ T cells recognize the antigenic peptide bound to major histocompatibility complex class II-antigens on antigen-presenting cells. Regulation of class II antigen expression on the surface of antigen-presenting cells is a critical step in the control of immune responses (Unanue et al., 1984). Human leukocyte antigen-DR and -DQ are the principal class II molecules operative in antigen-recognition by autoreactive CD4$^+$ T cells in multiple sclerosis patients (Martin et al., 1990; Ota et al., 1990; Pette et al., 1990). Blockade of the interferon-γ-induced increases in human leukocyte antigen-DR and -DQ expression on antigen-presenting cell by glatiramer acetate could be one of the mechanisms which controls the deleterious effects of interferon-γ in multiple sclerosis. We have shown that glatiramer acetate blocks the increases in human leukocyte antigen-DR and -DQ expression induced by lipopolysaccharide and interferon-γ treatment of THP-1

cells. Two further steps are needed to determine the significance of this effect. It must be shown that glatiramer acetate has a similar effect on stimulated class II antigen expression on human monocytes and macrophages and that antigen presenting function is inhibited.

The major cell wall component of Gram-negative bacteria, lipopolysaccharide, is a potent activator of macrophage responses involved in the host defense against infection (Adams and Hamilton, 1992; Rietschel and Brade, 1992). Interferon-γ, a T cell-derived cytokine, also regulates monocyte activation and effector function including modulation of major histocompatibility complex antigen expressions (Li et al., 1994; Li and Bever, 1996). Activated monocytes produce cytokines such as interleukin-1β and tumor necrosis factor-α (Adams and Hamilton, 1992; Li et al., 1994), which are likely to contribute to myelin damage and local inflammation (Selmaj and Raine, 1988; Durum and Oppenheim, 1989). Tumor necrosis factor-α regulates immune responses (Old, 1987), damages myelin-forming oligodendroglia (Selmaj and Raine, 1988; Soliven et al., 1991), and stimulates astrocyte proliferation in vitro (Selmaj et al., 1990). Interleukin-1 and tumor necrosis factor-α activate T cells and mediate the perivascular demyelination and gliosis characteristic of multiple sclerosis plaques (Hofman et al., 1989; Selmaj et al., 1990). Glatiramer acetate has differential effects on cytokine expression in our system, with inhibition of tumor necrosis factor-α and enhancement of interleukin-1β. The exact mechanism for this effects is not clear. However, tumor necrosis factor-α has shown to amplify major histocompatibility complex class II gene induction (Chang and Lee, 1986; Arenzana-Seisdedos et al., 1988), while interleukin-1β downregulates it (Johnson et al., 1989; Smith et al., 1993), the effect of glatiramer acetate on these cytokines correlates with its effect on human leukocyte antigen-DR and -DQ. However, other factors may be involved because no change was seen in class II antigen expression at 5 μg/ml glatiramer acetate, a concentration which increased IL-1β production.

Cathepsin B (EC 3.4.22.1), a lysosomal cysteine proteinase, is implicated in myelin basic protein degradation (Yanagisawa et al., 1984). It is increased in the peripheral blood monocytes and macrophages of multiple sclerosis patients (Bever, 1991; Bever and Garver, 1992a,b; Bever et al., 1994), and in multiple sclerosis lesions (Bever and Garver, 1992a,b, 1995), and is induced in macrophages after interferon-γ treatment (Cieri and Bever, 1990). It is possible that during multiple sclerosis exacerbations, macrophages in multiple sclerosis lesions are activated by interferon-γ and produce proteinases which participate in demyelination and result in the fragments of myelin basic protein which are found in the cerebr spinal fluid of multiple sclerosis patients. In addition, the role of these nzymes might not be limited to th degradation of extracellular matrix (Barrett and Kirschke, 1981; Maciewicz and Wotton, 1991; Cardozo et al., 1992), but also may be related t the presentation of antigen to the major hi compatibility complex class II (Matsunaga et al., 1993) this regard, it has been shown that cathepsin B has a in cleaving invariant chains from class II major histoc patibility complex molecules (Reyes et al., 1991; Rc and Cresswell, 1991; Daibata et al., 1994; Mizuochi et 1994) and a role in the processing and presentation f c II major histocompatibility complex-restricted antig (Reyes et al., 1991; Matsunaga et al., 1993). Therefor is possible that cathepsin B participates in the proces and presentation of myelin basic protein peptides to immune system and enhances immune response. G ramer acetate inhibits the increases in cathepsin B acti induced by lipopolysaccharide and interferon-γ and c indirectly interfere with antigen presentation and T activation. This mechanism could contribute to the el of glatiramer acetate on multiple sclerosis disease acti

In summary, we have shown a downregulating ef with glatiramer acetate on lipopolysaccharide and in feron-γ-induced THP-1 cell human leukocyt antigen- and -DQ expression, cathepsin B activity, and tu necrosis factor-α production. Our findings suggest glatiramer acetate could downregulate class II hu leukocyte antigen expression and tumor necrosis facto production and decrease cathepsin B activity associ with monocytes and macrophages in vivo. These find suggest that the benefit of glatiramer ac tate seer clinical trials in multiple sclerosis patients could in par due to an effect on monocytes and macrophages. Fur studies using human monocytes and macrophages examining additional cytokines implicated in multiple s rosis will be needed.

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